

IN THE CLAIMS

On page 70 of the copy of the publication, the amendments to the listing of claims serves to replace prior versions of claims from its related international application.

Listing of claims

1. (Currently amended) An assembly comprising a gas-filled microvesicle bearing a first overall net charge and a component associated ~~to~~ with said microvesicle wherein said component bears a second overall net charge opposite in sign to said first net charge, comprises a biocompatible surface active agent and has a diameter of 100 nm or lower.
2. An assembly according to claim 1 wherein said associated component has a diameter of 80 nm or lower.
3. An assembly according to claim 1 wherein said associated component has a diameter of 50 nm or lower.
4. An assembly according to claim 1 wherein said associated component comprises a targeting ligand, a bioactive agent, a diagnostic agent or any combination thereof.
5. An assembly according to claim 4 further comprising a second component bearing an overall net charge, optionally comprising a different targeting ligand, bioactive agent, diagnostic agent or any combination thereof.
6. An assembly according to claim 5, wherein said second component bears an overall net charge equal in sign with respect to the charge of the microvesicle.
7. An assembly according to claim 1 wherein said biocompatible surface active agent is an amphiphilic material.
8. An assembly according to claim 1 wherein said biocompatible surface active agent is selected among (C₂-C₁₀) organic acids, organic fatty acids comprising a (C₁₂-C₂₄) aliphatic chain, pharmaceutically acceptable salts thereof, esters thereof with polyoxyethylene; polyionic (alkali) salts; organic amines; amides; quaternary amine salts; aminoacids; phospholipids; ; esters of mono- or oligo-saccharides with

(C₁₂-C₂₄), organic fatty acids; organic sulfonates; perfluoroorganic acids; polymeric surfactants; and mixtures thereof.

9. An assembly according to claim 1 wherein the ratio between the number of charges per mole of microvesicles and the number of charges per mole of the second component is from about 10:1 to about 1:10.

10. An assembly according to claim 9 wherein said ratio is of about 3:1 or less.

11. An assembly according to claim 9 wherein said ratio is of about 2:1 or less.

12. An assembly according to claim 1 wherein said microvesicle is a microbubble stabilized by an envelope comprising an amphiphilic film-forming compound or a microballoon having a material envelope.

13. (Currently amended) An assembly according to claim 12 wherein said amphiphilic film-forming compound ~~comprised in the envelope stabilizing the microbubble~~ is a phospholipid.

14. An assembly according to claim 13 wherein said envelope comprises a phospholipid or a lipid bearing a positive or negative net charge.

15. An assembly according to claim 14 wherein said phospholipid or lipid is selected from phosphatidylserine derivatives, phosphatidic acid derivatives, phosphatidylglycerol derivatives, polyethyleneglycol modified phosphatidylethanolamines, ethylphosphatidylcholine derivatives and the respective lyso-forms; cholic acid salts; deoxycholic acid salts; glycocholic acid salts; (C₁₂-C₂₄) fatty acid salts thereof; alkylammonium salts comprising at least one (C₁₀-C₂₀) alkyl chain; tertiary or quaternary ammonium salts comprising at least one (C₁₀-C₂₀) acyl chain linked to the nitrogen atom through a (C₃-C₆) alkylene bridge; and mixtures thereof.

16. An assembly according to claim 12 wherein the material envelope of said microballoon comprises a polymeric material, a proteinaceous material, a water insoluble lipid or any combination thereof.

17. (Currently amended) An assembly according to claim 12 ~~or 13~~ wherein the material envelope of said microballoon comprises an ionic biodegradable polymer[[s]].

18. An assembly according to claim 13 wherein the material envelope of said microballoon further comprises a phospholipid or a lipid bearing a positive or negative net charge.

19. An assembly according to claim 18 wherein said phospholipid or lipid is selected from phosphatidylserine derivatives, phosphatidic acid derivatives, phosphatidylglycerol derivatives, polyethyleneglycol modified phosphatidylethanolamines, ethylphosphatidylcholine derivatives and the respective lyso-forms; cholic acid salts; deoxycholic acid salts; glycocholic acid salts; (C₁₂-C₂₄) fatty acid salts thereof; alkylammonium salts comprising at least one (C₁₀-C₂₀) alkyl chain; tertiary or quaternary ammonium salts comprising at least one (C₁₀-C₂₀) acyl chain linked to the nitrogen atom through a (C₃-C₆) alkylene bridge; and mixtures thereof.

20. (Currently amended) An assembly according to ~~anyone of~~ claim 1 ~~to 6~~, wherein said component associated ~~to~~ with said microvesicle is a micelle.

21. An assembly according to claim 20 wherein said micelle comprises a polyethyleneglycol modified phospholipid; an alkylammonium salt comprising at least one (C₁₀-C₂₀) alkyl chain; a tertiary or quaternary ammonium salt comprising at least one (C₁₀-C₂₀) acyl chain linked to the nitrogen atom through a (C₃-C₆) alkylene bridge; a (C₁₂-C₂₄) fatty acid salt; a polymeric surfactant; or mixtures thereof.

22. An assembly according to claim 20 wherein said micelle comprises a (C₁₂-C₂₄) fatty acid di-esters of phosphatidylcholine, ethylphosphatidylcholine, phosphatidylglycerol, phosphatidic acid, phosphatidylethanolamine, phosphatidylserine or sphingomyelin.

23. An assembly according to claim 20 wherein said micelle comprises a phospholipid or a lipid bearing a positive or negative net charge, or a polymeric ionic surfactant.

24. An assembly according to claim 23 wherein said phospholipid or lipid is selected from phosphatidylserine derivatives, phosphatidic acid derivatives, phosphatidylglycerol derivatives, polyethyleneglycol modified phosphatidylethanolamines, ethylphosphatidylcholine derivatives and the respective lyso-forms; cholic acid salts; deoxycholic acid salts; glycocholic acid salts; (C₁₂-C₂₄) fatty acid salts thereof; alkylammonium salts comprising at least one (C₁₀-C₂₀) alkyl chain; tertiary or quaternary ammonium salts comprising at least one (C₁₀-C₂₀) acyl chain linked to the nitrogen atom through a (C₃-C₆) alkylene bridge; and mixtures thereof.

25. (Currently amended) An assembly according to claim 1 wherein said component associated to with said microvesicle is a colloidal nanoparticle.

26. (Currently amended) An assembly according to claim 1 wherein said component associated to with said microvesicle is a solid polymeric nanoparticle.

27. (Currently amended) An aqueous suspension of a physiologically acceptable liquid comprising an assembly according to any one of ~~the~~ claims 1 to 26 or 4.

28. (Currently amended) An assembly according to ~~any of the claim~~[[s]] 1 to 26, wherein an aqueous suspension of said assembly in a pharmaceutically acceptable carrier shows a ζ -potential which is decreased of at least 50% in absolute value with respect to the ζ -potential of an aqueous suspension in the same carrier of the gas-filled microvesicles forming said assembly.

29. An assembly according to claim 28 wherein said ζ -potential is decreased of at least 75% in absolute value.

30. An assembly according to claim 28 wherein said ζ -potential is decreased of about 100% or more in absolute value.

31. A pharmaceutical kit which separately comprises:

- a) a gas-filled microvesicle, or a precursor thereof, bearing a first overall net charge as a first component;
- b) a second component, or a precursor thereof, associable with said microvesicle bearing a second overall net charge opposite in sign to said first net charge, said associated component having a diameter of 100 nm or lower.

32. A pharmaceutical kit according to claim 31 further comprising a pharmaceutically acceptable liquid carrier.

33. A pharmaceutical kit according to claim 32 wherein said first and second components are in the form of separate freeze-dried preparations.

34. A pharmaceutical kit which comprises:

- a) a gas-filled microvesicle, or a precursor thereof, bearing a first overall net charge as a first component;

b) a second component, or a precursor thereof, associated with said microvesicle bearing a second overall net charge opposite in sign to said first net charge, said associated component comprising a biocompatible surface active agent and having a diameter of 100 nm or lower.

35. (Currently amended) A ~~M~~method for preparing an assembly according to ~~any of the previous~~ claim[[s]] 1 to 26, which comprises admixing a preparation comprising gas-filled microvesicles or a precursor thereof with a preparation comprising a component or a precursor thereof to be associated to said microvesicles.

36. (Currently amended) A ~~M~~method according to claim 35 which comprises:

- 1) preparing a first aqueous suspension comprising a gas-filled microvesicle;
- 2) preparing a second aqueous suspension comprising a component to be associated with said gas-filled microvesicle;
- 3) admixing said two suspensions, to obtain an aqueous suspension comprising said assembly.

37. (Currently amended) A ~~M~~method according to claim 35 which comprises:

- 1) preparing a first aqueous suspension comprising a gas-filled microvesicle;
- 2) freeze-drying said suspension, to obtain a first lyophilized product;
- 3) preparing a second suspension comprising a component to be associated with said gas-filled microvesicle;
- 4) freeze-drying said suspension, to obtain a second lyophilized product;
- 5) reconstituting said first and said second lyophilized product with a physiologically acceptable aqueous carrier in the presence of a gas, to obtain an aqueous suspension comprising the assembly.

38. (Currently amended) A ~~M~~method according to claim 37, wherein step 5) comprises the steps of:

- a) reconstituting the second lyophilized product with a physiologically acceptable aqueous carrier to obtain a suspension comprising the component to be associated to the gas-filled microvesicle; and
- b) reconstituting the first lyophilized product with said suspension in the presence of a gas.

39. (Currently amended) A ~~M~~method according to claim 35 which comprises:

- 1) preparing an aqueous emulsion comprising an organic solvent, a phospholipid and a lyoprotecting agent;
- 2) preparing an aqueous suspension comprising a component to be associated with a gas-filled microvesicle;

- 3) admixing said aqueous suspension with said aqueous emulsion; and
- 4) freeze drying the mixture to remove the water and the organic solvent, to obtain a lyophilized product comprising said assembly.

40. (Currently amended) A Mmethod for preparing an assembly of claims 6 which comprising a gas-filled microvesicle bearing a first overall net charge and a component associated with said microvesicle wherein said component bears a second overall net charge equal in sign to said first net charge, comprises a biocompatible surface active agent and has a diameter of 100 nm or lower, wherein said method comprises admixing a the second component[[,]] with bearing an overall net charge equal in sign with respect to the charge of the gas-filled microvesicles, to the assembly obtained according to any of the claim[[s]] 35 to 39.

41. (New) An assembly according to claim 20 wherein said component comprises a targeting ligand, a bioactive agent , a diagnostic agent or any combination thereof.

~~42.~~ 41 (Currently amended) Use of A pharmaceutically active formulation comprising an assembly according to any one of the claims 1,4,20 or 41. to 29 for preparing a pharmaceutically active formulation.

~~43.~~ 42 (Currently amended) A method for ultrasound diagnostic imaging which comprises administering a contrast-enhancing amount of an aqueous suspension of an assembly according to any one of the claims 1, to 26, 4, 20 or 41.

~~44.~~ 43 (Currently amended) A method of therapeutic treatment which comprises administering a therapeutically-effective amount of an aqueous suspension of an assembly comprising a bioactive agent as defined in any one of the claims 1 to 26 further comprising a bioactive agent 4 or 41.

45. (New) An aqueous suspension of a physiologically acceptable liquid comprising an assembly according to any one of claims 20 or 41.